Volume 15 Number 1 1987 Nucleic Acids Research

Sequence dependent electrophoretic mobilities and melting temperatures for A·T containing oligodeoxyribonucleotides

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Received August 20, 1986; Revised and Accepted December 1, 1986

ABSTRACT

The electrophoretic mobilities and thermal melting properties of self complementary A·T containing dodecamer oligodeoxyribonucleotides have been investigated as a function of solution conditions. The oligomers contained tracts of nonalternating A·T base pairs of 2 ($d(A_2T_2)_3$), 3 ($d(A_3T_3)_2$), and 6 ($d(A_6T_6)$) as well as the fully alternating ($d(A-T)_6$) sequence. The melting temperature increased with the length of the nonalternating sequence and was approximately 12°C higher in the $d(A_6T_6)$ sequence than in the alternating oligomer. Under denaturing conditions all oligomers had the same electrophoretic mobility on acrylamide gels. Under conditions which favor duplex formation, the oligomers exhibited significant sequence dependent mobility differences. The mobilities of two oligomers, $d(A-T)_6$ and $d(A_6-T_6)$, were approximately equal and were less than those of the other oligonucleotides. The greatest mobility was observed for $d(A_2T_2)_3$. These results are best explained by a model which requires bending at a junction of two or more continuous A or T bases with another sequence.

INTRODUCTION

DNA sequences containing lengths of nonalternating A·T base pairs have been shown to exhibit unusual structural (reviewed in 1, 2-6) and interaction (7-13) properties. On the basis of X-ray fiber diffraction results, Arnott and co-workers (2) have proposed an unusual heteronomous structure for Poly dA·Poly dT in which the A chain has the deoxyribose ring in the C3'-endo conformation, characteristic of A-form helical structures, while the sugar of the T chain is in the C2'-endo conformation more typical for B-form helices. In contrast, proton NMR NOE experiments have indicated that the Poly dA·Poly dT is in a fairly standard right-handed B conformation with the sugars on both chains primarily in the C2'-endo configuration (14,15). Recent Raman spectral results have indicated that, while Poly dA·Poly dT may contain some sugars in the C3'-endo configuration, this was a characteristic of all natural and synthetic polymer DNAs examined (16).

Gel electrophoresis and other hydrodynamic experiments have convincingly demonstrated that nonalternating A·T sequences can induce significant curva-

ture in DNA fragments (2-6, earlier literature reviewed in 1). Using polymerized synthetic oligonucleotides, Crothers and co-workers (3) have defined the key parameters in systematic DNA bending induced by nonalternating A·T sequences. They found that (i) the A·T tracts must be in phase with the helical turn of DNA in order to add coherently; (ii) interrupting the runs of A and T with another base pair decreased the observed long range bending; (iii) the length of the tract was important for the magnitude of bending obtained; and (iv) bending is more pronounced at the 3' than at the 5' end of an A·T tract. They concluded that a bend of approximately 9° exists between nonalternating A·T sequences and adjacent sequences in a B form helix (4).

There have also been several recent indications that the interaction properties of nonalternating A·T tracts are unusual. We have found that the intercalator propidium binds with positive cooperativity, a large positive enthalpy and positive entropy to Poly dA·Poly dT (11-13). Its binding to the alternating A·T polymer, on the other hand, is very similar to its interaction with natural DNA samples and G·C containing polymers (11-13). The outside binding compound netropsin also exhibits unusual thermodynamics in its interaction with Poly dA·Poly dT (10) and other intercalators exhibit positive cooperativity and/or weak binding with this polymer (7-9).

It should be emphasized that these unusual structural and cooperative interaction effects exist under physiological conditions of salt, temperature and pH and, therefore, nonalternating A·T sections in chromosomes should exhibit these properties. It has been shown, for example, that nonalternating A·T sections modify nuclease action on DNA (17) and that nucleosomes will not form on nonalternating A·T sequences (18). These sequences, thus, have the ability under normal cellular conditions to exert significant effects on chromosome structure and function.

Modifications of structure and interactions at the local (e.g., one to two turn) level will be most important if sections of nonalternating A·T are to have an effect on gene function in vivo. For this reason we have begun investigating the microscopic structure and interactions of oligomers containing only A·T base pairs (Scheme 1). We have shown that $d(A\text{-}T)_6$, a dodecamer with alternating A·T base pairs, behaves much like the analogous alternating polymer and natural DNA in its interaction with propidium iodide (13). Oligomers containing nonalternating A·T sequences (Scheme 1), however, were found to exhibit interaction properties much more similar to Poly dA·Poly dT (13).

It is important to investigate microscopic sequence dependent variations in the structure of these oligomers and, as a first step, we report here an

analysis of their gel electrophoretic behavior. When conducting electrophoretic studies on oligonucleotides, the thermal stability of the duplexes under the electrophoresis conditions is a critical consideration. For this reason we have also determined, and report here, Tm curves for the oligomers of Scheme 1. The principal findings of these experiments are that the A·T sequence oligomers have significant sequence dependent differences in Tm values and electrophoretic mobilities.

EXPERIMENTAL

Oligomers

Oligomers were synthesized, purified and characterized as previously described (13,19).

Tm Determinations

Oligomer UV spectral analysis and melting experiments were performed on a Cary 219 spectrophotometer interfaced to an Apple IIe microcomputer. Up to 5 cells were monitored and thermostatted in a 5-position rotatable cell turret. The temperature control was through a Haake PG20 temperature programmer connected to a Haake A81 refrigerated water bath. Temperature was monitored by a Cary 219 thermistor unit with the thermistor sealed in a reference cuvette in one position of the turret. Other cuvettes had Teflon stoppers and all were 1-cm pathlength quartz cells. Nitrogen gas was passed continuously through the sample compartment of the Cary 219 during low temperature measurements. Digitized absorbance values were plotted as a function of temperature on the Cary 219 chart recorder and were simultaneously stored by the computer for subsequent plotting and analysis. The computer collected and averaged 10 absorbance readings for each point on the Tm curve to improve signal-to-noise. Tm measurements were initiated near 0°C and the temperature ramp was 0.5°C/min. For the relatively broad Tm curves obtained for the DNA oligomers, adsorbance readings were collected only once per min, and this typically resulted in more than 100 points for a melting curve and baseline. The digitized results were fitted with a nonlinear least squares program using the methods of Turner and co-workers (20). The program fitted the melting curve with temperature dependent extinction coefficients for both the helix and single strand regions and best fit values for the Tm and apparent enthalpy and entropy for the melting transition. PIPES 20 buffer used in the Tm experiments contained 10 mM PIPES, 1 mM EDTA, and 0.2 M NaCl at pH 7.

Electrophoresis

Electrophoresis was carried out under duplex conditions as described by

Maniatis et al (21) and under denaturing conditions as described by Frank and Köster (22). 20% polyacrylamide gels (30:0.8 acrylamide:bisacrylamide) were prepared in a Biorad Protean II gel apparatus using 0.75 mm spacers. Nondenaturing gel experiments were conducted with TBM buffer (0.1 M Tris-borate, 0.005 M $MgCl_2$, pH 8.3). Denaturing gels contained 7 M urea and were run in TBE buffer (0.1 M Tris-borate, 0.02 M EDTA, pH 8.3). Oligomers (20 A_{260} units/ml) were diluted 1:10 with gel loading buffer (TBE containing 0.05% bromophenol blue plus 8% sucrose) and applied to the gels in 2-4 µl volumes. Non-denaturing gels were run at 3-5 mA for 16-20 hours. Denaturing gels were run at 15 mA for 3 hours. In both cases, electrophoresis was stopped when the bromophenol dye had migrated approximately 12 cm. Gels were then stained with propidium iodide for 5 minutes and photographed under uv light using a Polaroid MP-4 Land Camera equipped with a Wratten #9 filter.

RESULTS

To determine the relative duplex stability for the oligomers shown in Scheme 1, we measured melting curves in PIPES 20, a standard buffer system we

Scheme I.

Oligomer Sequence	Abbreviation
dATATATATATAT	$d(A\text{-}T)_6$
dAATTAATTAATT	$d(A_2T_2)_3$
dAAATTTAAATTT	$d(A_3T_3)_2$
dAAAAAATTTTTT	$d(A_6T_6)$
dCGCGAATTCGCG	D

have used for comparative characterization of oligomers (23), and in the two Tris-borate buffers used for electrophoresis. We also conducted electrophoresis as a function of temperature to relate the mobilities on gels to the duplex-strand equilibrium.

Tm Determinations

Comparison of the melting curves for the four A·T sequence oligomers in PIPES 20 buffer (Figure 1) indicates that the oligomers, although of identical base composition, exhibit considerable sequence dependent variations in the Tm values. The highest Tm is for the dA_6T_6 oligomer, which contains the smallest number of alternating A·T base pairs. The lowest Tm is for the $d(A\text{-}T)_6$ oligomer which is fully alternating and intermediate Tm values are observed for

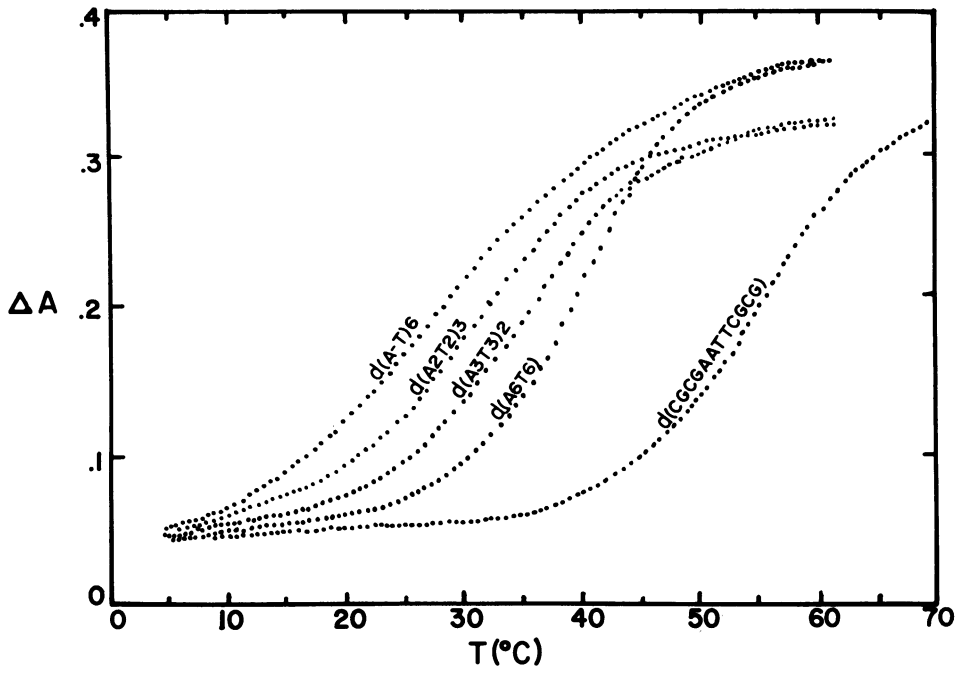

Figure 1. Melting curves in PIPES 20 buffer for the oligomers of Scheme 1. The absorbance changes at 260 nm are plotted as a function of temperature. The oligomer concentration in all cases was near 1.5×10^{-4} M in bases.

$d(A_2T_2)_3$ and $d(A_3T_3)_2$. The Tm curves have similar shapes but the curve for dA_6T_6 is steeper, for example, than the Tm curve for $d(A\text{-}T)_6$, indicating a lower transition enthalpy for the $d(A\text{-}T)_6$ oligomer. Fitting the two limit curves by the method of Turner and co-workers (20) yields a transition enthalpy of 47 kcal/mole for $d(A\text{-}T)_6$ and 78 kcal/mole for dA_6T_6.

A similar order of Tm values is observed in electrophoresis buffers. Curves for the four A·T sequence oligomers in TBM buffer (Figure 2) are slightly sharper than in PIPES 20. The Tm values are in the same order and have values similar to those in PIPES 20. As expected, the Tm values for all oligomers are markedly lower in TBE buffer (not shown). The Tm of dA_6T_6, for example, decreases by over 15°C in going from TBM to TBE buffer. The melting curves, particularly for $d(A\text{-}T)_6$, are quite broad in TBE and other low salt buffers. It is quite possible that at the higher temperatures and in the low salt buffers some hairpin conformation, of the type seen by Baldwin and co-workers for $d(T\text{-}A)_n$ type oligomers (24), forms and causes the observed

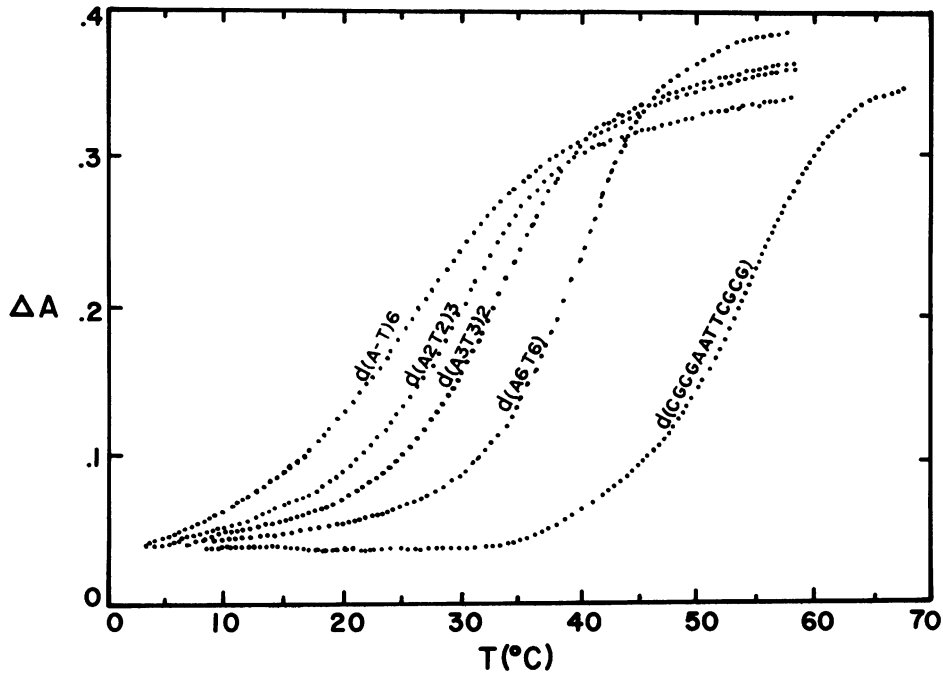

Figure 2. Melting curves in TBM (5 mM Mg ion) buffer for the oligomers of Scheme 1. Other conditions are as in Figure 1.

broadening of the Tm curves. Under very low ionic strength conditions ([Na^+] < 0.01 M) biphasic Tm curves, which are characteristic of duplex to hairpin and hairpin to strand transitions (24), are observed. As the ionic strength is increased, the curves become monophasic, sharper and completely reversible as expected for duplex to strand transitions.

There is no evidence for multiphasic transitions for any of these samples in any of the high ionic strength buffers, suggesting that hairpin or other types of structures, intermediate between the duplex and single strands, are not present in significant amounts. We conclude from these experiments that electrophoretic mobilities of these oligomers observed on gels with TBM buffer at low temperature will be characteristic of duplex oligomers. In contrast, gels with TBE buffer will have significant amounts of single strands and perhaps hairpins for oligomers such as $d(A-T)_6$, even at 0-4°C. The duplex to strand transition during electrophoresis can also be affected by raising the temperature of TBM gels to destabilize the duplex or, alternatively, by increasing the magnesium ion concentration to increase duplex stability.

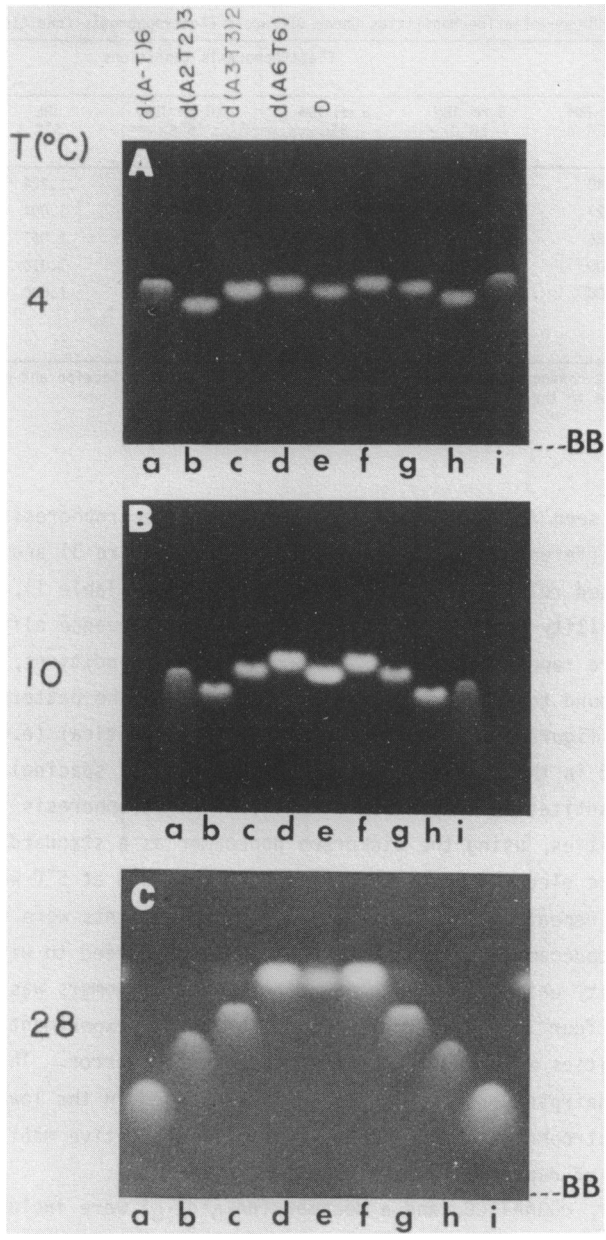

Figure 3. Electrophoresis in TBM (5 mM Mg ion) buffer as a function of temperature: A, 5°C; B, 10°C; C, 28°C. Lanes are: a, $d(A\text{-}T)_6$; b, $d(A_2T_2)_3$; c, $d(A_3T_3)_2$; d, $d(A_6T_6)$; e, D; f, $d(A_6T_6)$; g, $d(A_3T_3)_2$; h, $d(A_2T_2)_3$; and i, $d(A\text{-}T)_6$. BB indicates the position of the bromophenol blue dye in A and C. The dye position is slightly off the bottom of the photograph in B. Other electrophoresis conditions are described in the Methods Section.

Table I. Relative Oligonucleotide Mobilities Under Different Electrophoresis Conditions[a]

Oligomer	Electrophoresis Conditions					
	5 mM TBM 28°C	5 mM TBM 10°C	5 mM TBM 5°C	10 mM TBM 5°C	TBE 5°C	7 M urea TBE 28°C
$d(A\text{-}T)_6$	1.240	0.996	0.980	0.963	1.224	0.849
$d(A_2T_2)_3$	1.127	1.020	1.028	1.030	1.084	0.849
$d(A_3T_3)_2$	1.085	0.993	0.995	1.008	1.057	0.849
$d(A_6T_6)$	1.000	0.979	0.973	0.975	1.010	0.849
D	1.000	1.000	1.000	1.000	1.000	1.000
$dA_{10}\cdot dT_{10}$		1.109				
dGGAATTCC		1.209				

[a]Electrophoresis was conducted as described in the Materials and Methods Section and mobilities were calculated relative to the Dickerson dodecamer, D.

Electrophoresis

As can be seen from photographs of gels from electrophoresis experiments conducted at different temperatures in TBM buffer (Figure 3) and from mobilities, calculated relative to the Dickerson dodecamer (Table I), there are significant mobility differences among the four A·T sequence oligomers. These experiments were repeated at least two times for all conditions, and the results were found to be reproducible. In particular the patterns, such as those shown in Figure 3, were always qualitatively identical (e.g., oligomers always migrated in the same order with the same general spacing). In order to improve the quantitative comparison of oligomer electrophoresis results, relative mobilities, using the Dickerson dodecamer as a standard, were calculated. The electrophoresis experiment in 5 mM TBM at 5°C was quantitatively repeated four times (e.g., gels experiments were conducted with the Dickerson dodecamer present) and the mobilities agreed to within ±0.005 relative mobility units. The concentration of the oligomers was also varied by a factor of four in the low temperature TBM buffer experiment and the relative mobilities were constant within experimental error. This again suggests that hairpin formation is not a major factor in the low temperature TBM buffer electrophoresis experiments and the quantitative mobilities are characteristic of duplex oligomers.

An octamer, dGGAATTCC, and a decamer ($dA_{10}\cdot dT_{10}$) were included in the low temperature experiments in TBM buffer, and their mobilities are listed in Table I for reference. To increase the oligomer duplex stability, a low temperature electrophoresis experiment was also conducted in TBM type buffer containing 10 mM rather than 5 mM magnesium ion. As can be seen from Table I,

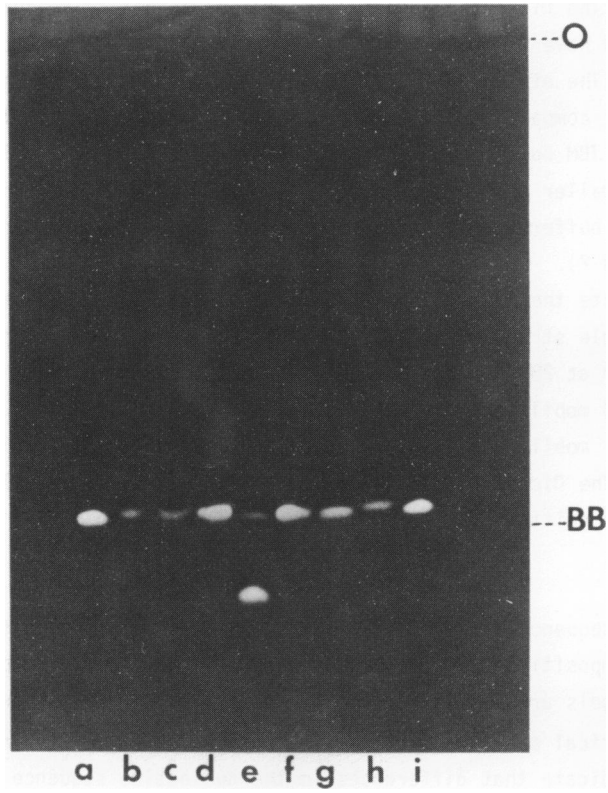

Figure 4. Electrophoresis in a 7 M urea-TBE gel at 28°C. Samples a-i are as in Figure 3 except that e was a mixture of D and $d(A_6T_6)$. The positions of the bromophenol blue dye, BB, and the origin, O, are indicated.

the migration pattern was in the same order as in 5 mM magnesium and only slight changes were observed in the relative mobilities when the magnesium ion concentration was increased to 10 mM. Significant changes were observed in relative mobilities on raising the temperature to 28°C. These changes were most striking for $d(A\text{-}T)_6$, which is consistent with the low melting temperature observed for this oligomer (Figure 2). Little change was observed for the relative mobility of dA_6T_6, again as expected from the Tm results (Figure 2).

The mobilities observed in an experiment conducted in TBE buffer at 5°C (Table I) were similar to those seen with TBM buffer at high temperature. This finding is also consistent with the Tm results, indicating that $d(A\text{-}T)_6$ contains significant amounts of single stranded oligomer in TBE even at low

temperature. The Dickerson and dA_6T_6 oligomers appear to be fully duplexed in TBE at 5°C and have very similar mobilities in all TBM and TBE buffer experiments. The effect of the duplex to strand transition on gel mobilities can be seen by comparing results for $d(A\text{-}T)_6$ in TBM buffers at low temperature to results in TBM buffers at high temperature (Figure 2, Table I) and in TBE (Table I). Smaller changes in mobility are seen for $d(A_2T_2)_3$ and $d(A_3T_3)_2$ in the different buffers, as would be predicted from their intermediate Tm values (Figures 1 and 2).

To evaluate the relative mobilities of all of these dodecamers as denatured single strands, an electrophoresis experiment was conducted in TBE-7M urea buffer at 28°C. As can be seen from the gel results in Figure 4 and the calculated mobilities in Table I, all of the A·T containing dodecamers have identical mobilities within experimental error under denaturing conditions. The Dickerson dodecamer has a higher relative mobility under denaturating conditions as expected from its composition (22).

DISCUSSION

The A·T sequence dodecamers illustrated in Scheme 1, even though of identical base composition, exhibit dramatic differences in electrophoretic properties when gels are run under conditions that favor duplex formation (Figure 2). The identical mobilities of these oligomers under denaturing conditions (Figure 4) indicate that differences in the nucleotide sequence and interaction with the gel matrix do not have a significant effect on their migration. Thus, there is nothing intrinsic to the strand sequence of these dodecamers that could account for the observed differences in duplex mobility.

The variation in mobilities seen in Figure 2 could arise from two primary causes: (1) different mixtures of duplex and single strands in rapid enough equilibrium to affect average gel mobility and/or (2) differences in duplex structure. It is the latter point which is of the most interest and which we have begun to probe by electrophoresis studies. To probe conformation, it is necessary that the duplex to strand equilibrium be convincingly shifted to the duplex state. The Tm curves in Figure 2 illustrate that this occurs in TBM buffer at low temperature. Once the duplex state is reached, increasing magnesium ion concentration should have a small effect on relative mobilities as observed (Table I). As the temperature is raised or if the electrophoresis buffer is switched to TBE, mobilities of the lower melting oligomers are strongly affected by the duplex to strand equilibrium. These experiments, thus, demonstrate that in TBM buffer at low temperature, the A·T

sequence oligomers of Scheme I migrate with their limiting duplex mobilities and that these mobilities vary significantly with sequence.

The observed mobility differences must then reflect sequence dependent microconformational differences at essentially the single turn level of the DNA double helix. As can be seen from Figure 3 and Table I, the intrinsic duplex mobilities (TBM buffer at low temperature) are very similar for $d(A\text{-}T)_6$ and dA_6T_6, the oligomers with the largest differences in sequence and Tm values. The oligomer $d(A_3T_3)_2$ moves relatively faster and $d(A_2T_2)_3$ moves faster still. These results suggest that at the microstructural level $d(A\text{-}T)_6$ and dA_6T_6 are similar while $d(A_3T_3)_2$ and $d(A_2T_2)_3$ are hydrodynamically more compact.

At the macromolecular level, it has been clearly shown that phasing of sequences which can cause bends is the dominant factor affecting electrophoresis gel mobilities (3-6). In phase bends add coherently to give significant macromolecular curvature and reduce the rate of migration through gels. When using oligomers of essentially a single turn of the double helix, however, no long range effects are possible and it is the fragment conformation which is of most importance in influencing gel mobilities. Only moderate nonlinearity of the helix axis can be obtained with small bends at the microscopic level and the number of bends should be the factor which most strongly determines the effective fragment conformation and, therefore, the rate of migration through gels. Each bend can decrease the effective length of the duplex oligomer and <u>increase</u> the mobility of the oligomer. This argument assumes that sequence induced bends will be small, e.g., in the 10° range, rather than large bends or kinks which will cause major structural changes in the double helix. This hypothesis is supported by the fact that only small bends are predicted from gel electrophoresis results with larger DNA molecules (3,25).

It is important to try to quantitate the observed mobility differences (Table I) in terms of measurable structural features of oligomers. This is difficult, however, since there is no well-defined theory for interpreting gel mobilities in electrophoresis experiments in terms of duplex conformation. In TBM buffer the mobility difference between the Dickerson dodecamer and a decamer is 10-11%, and there is another change of approximately 10% in going to an octamer (Table I). Although a logarithmic relationship is expected, these results suggest an approximately 5% change in mobility per base pair added to the double helix over this narrow range. The difference between the mobilities for dA_6T_6 and $d(A_2T_2)_3$ is approximately 5%, suggesting that the

conformational differences between these oligomers result in structures which at the single turn level behave as if they differ in length by about one charged base pair. The difference between $d(A_3T_3)_2$ and dA_6T_6 is approximately one-half as much. These numbers can not be directly interpreted in terms of absolute length changes, however, since helix charge and helix diameter contribute to the observed electrophoretic mobilities. Increasing the helix length without increasing the charge will obviously not have the same effect on mobility as increasing the length by adding a charged base pair.

As has been pointed out by numerous investigators, bends can occur in the double helix at nonalternating A·T sequences (1,3-6). Interestingly, however, totally alternating A·T sequences do not appear to have any significant sequence dependent bending (1) or unusual interaction properties (10-13). The $d(A\text{-}T)_6$ oligomer would, thus, be expected to have no intrinsic curvature. Predicting the curvature of the other A·T sequence isomers requires the choice of a model among those proposed to account for bending in A·T sequences: 1) the ApA wedge model (1), 2) the purine clash model of Calladine (26) and Dickerson (27), 3) the refined purine clash model of Tung and Harvey (28,29) which includes conformational energy calculations and 4) junction-bending models which have been described in detail by Crothers and co-workers (3,4 and references therein). The first three of these models have been tested for these oligomers using a molecular graphics computer program supplied by Dr. S. Harvey (29). We find that the structure predicted by these three models would not be expected to exhibit the relative electrophoretic mobilities that we observed (Table I). The ApA wedge model (1) predicts extensive bending for $d(A_6T_6)$ and no bending for $d(A\text{-}T)_6$. According to this model $d(A\text{-}T)_6$ and dA_6T_6 would have the most significant mobility differences in the duplex state with $d(A_2T_2)_3$ and $d(A_3T_3)_2$ falling in between. As can be seen from the duplex results in Table I, this model is not supported by our data. The purine clash model as described by Dickerson and co-workers (27) predicts no significant bending for any of the oligomers. The model of Tung and Harvey (28,29) is more successful at predicting bends, but the oligomer of Scheme 1 with the most significant predicted curvature is $d(A_3T_3)_2$ in contrast to the experimental results. In this model $d(A_2T_2)_3$ does not appear to have sufficient curvature to account for the duplex mobilities in Table I.

If it is assumed in the junction bending model that a bend will occur at runs of A of two or longer and that this is the most significant effect for the mobility differences of the A·T oligomers of Scheme I, then this model agrees well with the results of Table I. This is also the model which best

explains observed mobility results with synthetic DNA polymers containing nonalternating A·T sequences (3,4). According to the junction bend model dA_6T_6 would have one bend in the center and would, therefore, be expected to be hydrodynamically only slightly more compact than $d(A\text{-}T)_6$. In the low temperature 10 mM magnesium ion buffer, where the duplexes have the greatest stability, dA_6T_6 has a slightly higher mobility than $d(A\text{-}T)_6$ as predicted by the junction bend model. $d(A_3T_3)_2$, which has two 5' A-T 3' junctions and one 5' T-A 3' junction where bending can occur, has a higher mobility than either $d(A\text{-}T)_6$ or $d(A_6\text{-}T_6)$. $d(A_2T_2)_3$ has three 5' A-T 3' and two 5' T-A 3' junctions and has the highest mobility of any of the A·T series oligomers (Scheme I). The mobility differences, and presumably the sequence dependent bends, are observed over temperature and salt conditions that maintain duplex stability. All of these results are in agreement with a model in which slight bends are induced in the DNA duplex structure at A-sequences which contain two or more continuous A's. It should be noted, however, that there can be significant sequence dependent variations in DNA distortion caused by dAn sequences. Hagerman (6) has shown, for example, that in contrast to 5'dAnTn3' sequences, 5'dTnAn3' sequences do not cause significant bending of the double helix.

These results clearly illustrate that significant distortion of the double helix can occur where nonalternating A·T sequences join other DNA sequences and that sequences of two or more nonalternating A·T base pairs can have a pronounced effect on the local structure of the DNA duplex at the single turn level. Although these microstructural units will only affect long range conformation if they are in phase, they can have a pronounced effect on localized DNA structure and interactions. Since these unusual effects exist under normal cellular conditions, they should exert a significant influence on chromosome structure and function.

ACKNOWLEDGMENT

This work was supported by NIH Grant GM 30626 (W.D.W.), American Cancer Society Faculty Research Award (FRA-267) (W.D.W.), NSF Grant DMB-8402450 (B.R.B.) and NIH Grant RR07171 (B.R.B.). We thank Dr. S. Harvey for supplying the molecular graphics program used to analyze oligomer structure. We also thank the Referees for helpful comments.

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